Investigating subsurface microbial metabolisms using high-pressure microfluidic tools

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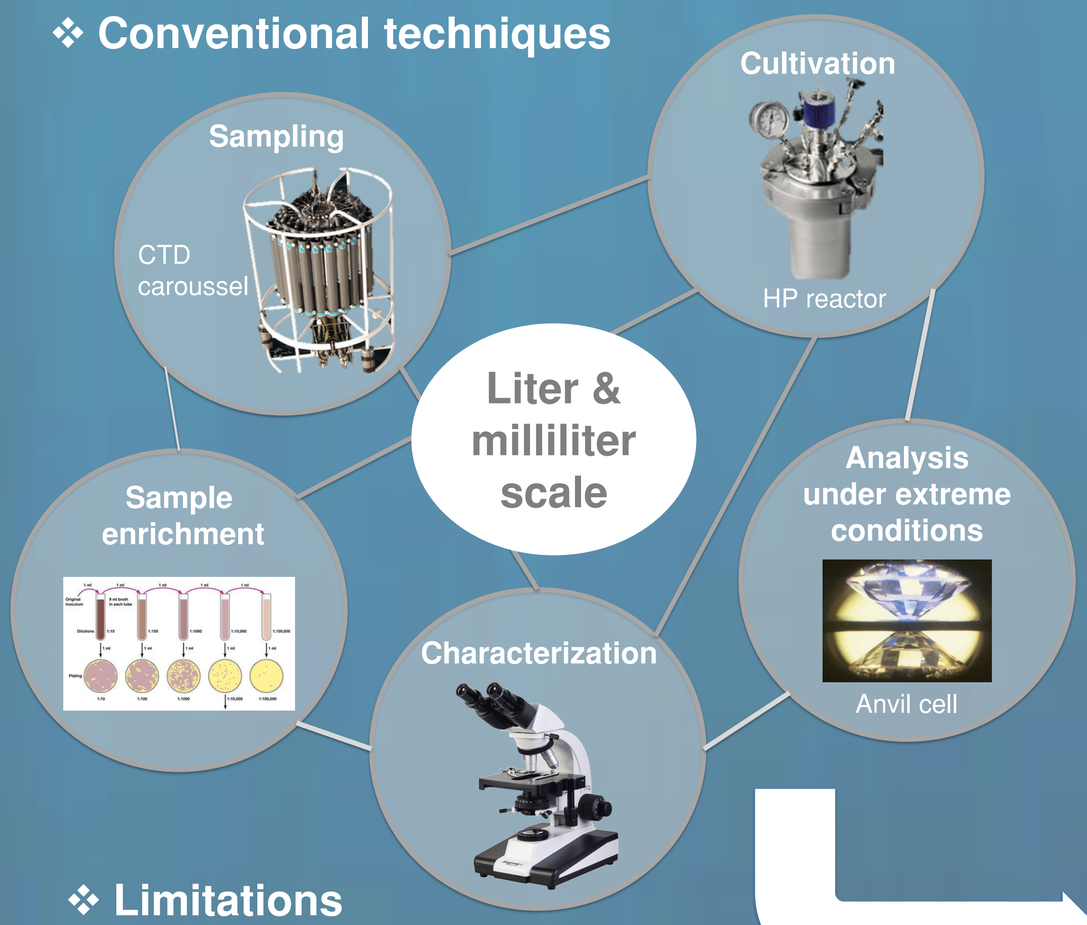
Context

❖ The deep biosphere : the unseen majority

A majority of Earth's prokaryotes resides in the deep biosphere (deep sea or deep underground environments) where little is known about how inherent elevated pressures impact the underground geochemistry and the inhabiting microbial communities (Jannash & Taylor, 1984). The deep biosphere represents the unseen majority (~ 99 %) of the total biosphere on Earth (Whitmann *et al.,* 1998 ; Bar-On *et al.,* 2018) and has gained increasing attention given its significance in a variety of critical processes and topics (carbon cycle, origins of life, biomining, CO_2 storage, etc).

❖ Conventional techniques



❖ Limitations

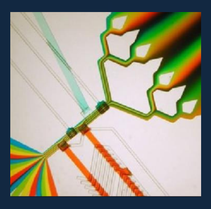
- Decompression of samples for analyses
- Limited control of the operating parameters (p, T, concentration, etc)
- Limited *in situ* characterization techniques

µl & nl scale

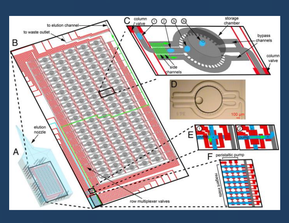
How can we use microfluidics to study the deep biosphere ?

Mimicking specific environments

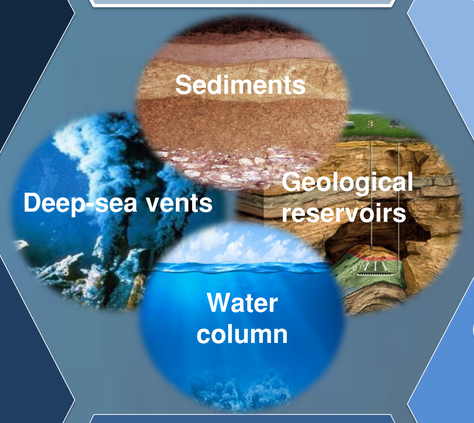
by reproducing several gradients



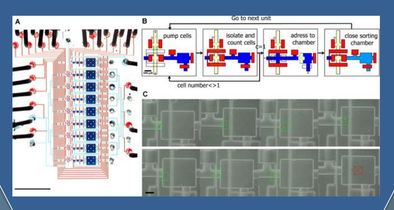
Cultivation of microbes in nanochambers



Leung *et al.,* 2012



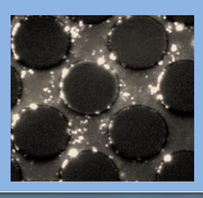
Single cell analysis



Marcy *et al.,* 2007

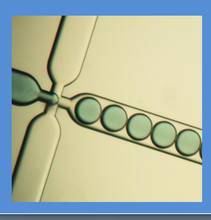
Reproducing physical characteristics

(*i.e.* pores geometry , porosity and permeability)



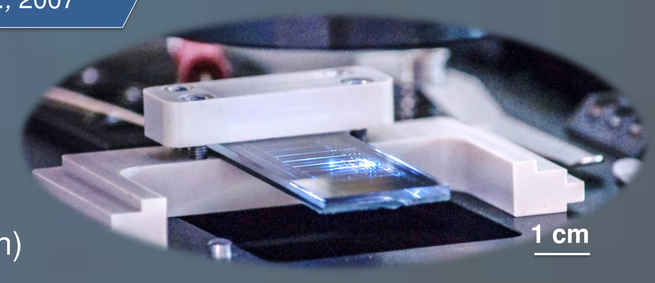
Isolation of new microorganisms

(cell encapsulation, cell sorting)
Droplet-based microfluidics



❖ Is small better ?

- High pressure capability (up to 40 MPa)
- Fast screening (vs. autoclave batch mode)
- Multiple on chip conditions (p, T, composition)
- Multi analyses within a single experiment (reproductibility)
- *In situ* and online characterization (Live imaging & process characterization)
- Flexible design (porous media, microchannels, etc.) (*vs.* capillaries and tubings)

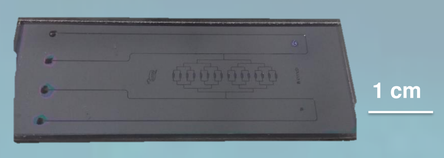


Microreactor

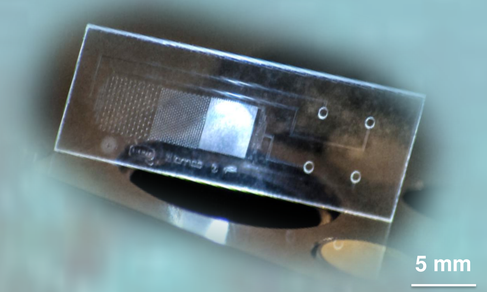
HP/HT Microfluidics at ICMCB

Technology

- Semi-transparent (Silicon - Pyrex)



- Transparent (glass, ...)

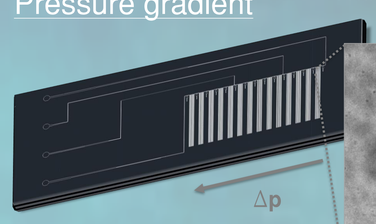


- Small volume : nl to µl

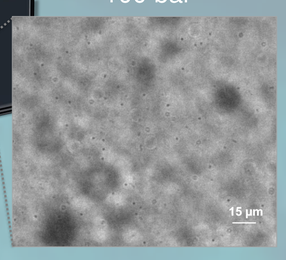
Fast screening of operating parameters

- ∆p : 1 bar - 400 bar
- ∆T : 0°C - 500°C
- ∆C : pH, salinity, ...

Pressure gradient

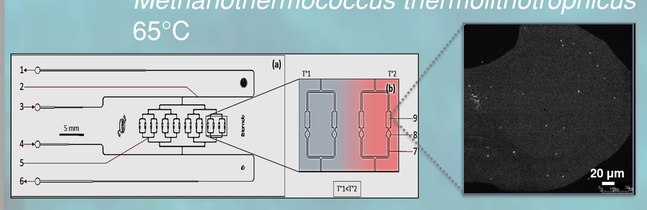


Thermococcus barophilus
100 bar



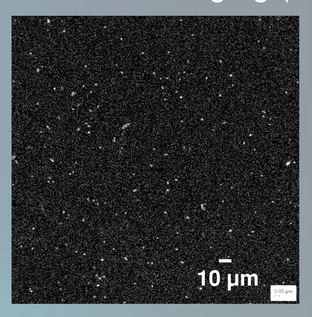
Temperature gradient

Methanothermococcus thermolithotrophicus
65°C



In situ characterization

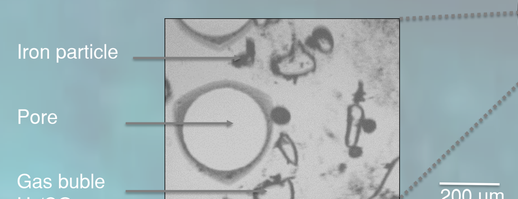
- Live imaging (confocal microscopy)



Photobacterium phosphoreum

Fluorescence microscopy
(λ_{ex} 405 nm)

- FTIR spectroscopy

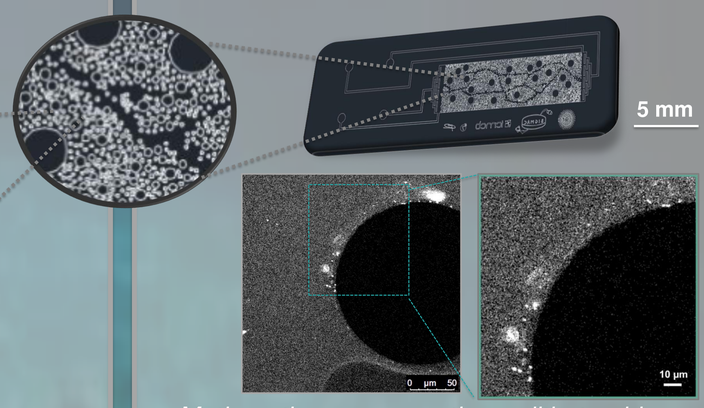


- Raman spectroscopy

Applications

- Growth monitoring
- Segmented flow / droplets
- Mimicking environments :

Porous medium within geological conditions (70 -200 bar / 30 – 100°C)



Methanothermococcus thermolithotrophicus

Conclusion and perspectives

- Microfluidic approaches are promising tools for exploring the deep biosphere at lab scale.
- New opportunities to increase the number of new isolates and discover uncultured microbes.
- Possible combination with *in situ* spectroscopic characterization techniques to monitor microbial activities and metabolisms (single-cell, PCR, SERS (Surface Enhanced Raman Spectroscopy) to detect low quantities of DNA, FISH, ...).

References

- Bar-On *et al.* (2018) PNAS 115(25): p. 6506-6511
- Jannasch and Taylor (1984) Annual Reviews in Microbiology 38(1): p. 487-487
- Kaston Leung *et al.* (2012) PNAS 109:20:7665-7670
- Marcy *et al.* (2007) PLOS Genetics 3(9): e155
- Whitman *et al.* (1998) PNAS 95(12): p. 6578-6583

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